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The Pentose Cycle and Insulin Release in Mouse Pancreatic Islets

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1. Rates of insulin release, glucose utilization (measured as [^{3}H]water formation from [5-^{3}H]glucose) and glucose oxidation (measured as $^{14}CO_2$ formation from [1-^{14}C]- or [6-^{14}C]-glucose) were determined in mouse pancreatic islets incubated *in vitro*, and were used to estimate the rate of oxidation of glucose by the pentose cycle pathway under various conditions. Rates of oxidation of [U-^{14}C]ribose and [U-^{14}C]xylitol were also measured. 2. Insulin secretion was stimulated fivefold when the medium glucose concentration was raised from 3.3 to 16.7mM in the absence of caffeine; in the presence of caffeine (5mM) a similar increase in glucose concentration evoked a much larger (30-fold) increase in insulin release. Glucose utilization was also increased severalfold as the intracellular glucose concentration was raised over this range, particularly between 5 and 11mM, but the rate of oxidation of glucose via the pentose cycle was not increased. 3. Glucosamine (20mM) inhibited glucose-stimulated insulin release and glucose utilization but not glucose metabolism via the pentose cycle. No evidence was obtained for any selective effect on the metabolism of glucose via the pentose cycle of tolbutamide, glibenclamide, dibutyryl 3′:5′-cyclic AMP, glucagon, caffeine, theophylline, ouabain, adrenaline, colchicine, mannoheptulose or iodoacetamide. Phenazine methosulphate (5μM) increased pentose-cycle flux but inhibited glucose-stimulated insulin release. 4. No formation of $^{14}CO_2$ from [U-^{14}C]ribose could be detected: [U-^{14}C]xylitol gave rise to small amounts of $^{14}CO_2$. Ribose and xylitol had no effect on the rate of oxidation of glucose; ribitol and xylitol had no effect on the rate of glucose utilization. Ribose, ribitol and xylitol did not stimulate insulin release under conditions in which glucose produced a large stimulation. 5. It is concluded that in normal mouse islets glucose metabolism via the pentose cycle does not play a primary role in insulin-secretory responses.

The present study is an investigation of the relationship between glucose metabolism by the pentose cycle and insulin release in mouse islets. The existence of an active pentose cycle in islets of Langerhans has been suggested on the basis of studies of the relative rates of $^{14}CO_2$ formation from [1-^{14}C]-glucose and [6-^{14}C]glucose by human islet tumour (Field *et al.*, 1960) and rat islets (Jarrett & Keen, 1966; Heinze & Steinke, 1971). Although such data do not permit a quantitative estimation of the flux of glucose via the pentose cycle (Katz & Wood, 1963) the potential capacity of islets of Langerhans to oxidize glucose by this route is indicated by the demonstration of the enzymes of this pathway in islets from several species (Ashcroft & Randle, 1970; Brolin *et al.*, 1964; Gepts & Toussaint, 1964; Matschinsky *et al.*, 1968). The observation that xylitol promotes insulin secretion in dogs (Kuzuya *et al.*, 1966; Hirata *et al.*, 1966) led to the suggestion (Montague & Taylor, 1970) that the pentose cycle might provide a signal for insulin release. In support of this idea, the effects of several agents on insulin release by rat islets could be correlated with their effects on the intraislet concentration of 6-phosphogluconate (Montague & Taylor, 1970). However, it was not established whether these changes were associated with variations in flux through the pentose cycle, nor is it known to what extent the pentoses that stimulate insulin release are metabolized by this route. Snyder *et al.* (1970) estimated the contribution of the pentose cycle to glucose metabolism in rat islets and found no increase when the medium glucose concentration was raised from 3.3 to 16.7mM.

To assess the importance of the pentose cycle in normal mouse islets we have measured the rate of metabolism of glucose by this pathway over a wide range of glucose concentrations and under a number of conditions known to modify rates of insulin release. In addition we have examined the metabolic and insulin-secretory activity of a number of pentoses and pentose derivatives.

Experimental

Materials

Collagenase, ribitol and xylitol were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., or from Boehringer Corp. (London) Ltd., London W.5, U.K. Glucagon was a gift from Eli Lilly and

Co., Indianapolis, Ind., U.S.A. Glibenclamide was a gift from Roussel Laboratories Ltd., Wembley Park, Middlesex, U.K. Bovine plasma albumin (fraction V) was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. Tolbutamide was a gift from Burroughs Wellcome and Co., London N.W.1, U.K. All radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. Other chemicals were from British Drug Houses Ltd., Poole, Dorset, U.K.

[5-^{3}H]Glucose, supplied as a solution in water (1mCi/ml, 2Ci/mmol) was dried *in vacuo* over P_2O_5 before use to remove any [^{3}H]water present. The [1-^{14}C]glucose used was that designated by The Radiochemical Centre as 'specially prepared substrate for enzyme assays' and was of low specific radioactivity (0.2mCi/mmol).

Methods

Preparation of islets. Islets were prepared from 3–4-week-old male white mice by a collagenase method previously described (Coll-Garcia & Gill, 1969). All incubations were carried out at 37°C in Krebs bicarbonate medium (Krebs & Henseleit, 1932) containing the additions given in the text or tables as previously described (Ashcroft *et al.*, 1970). Media containing phenazine methosulphate were kept in the dark throughout.

Output of $^{14}CO_2$. Batches of ten islets were incubated in 20μl of medium containing [1-^{14}C]- or [6-^{14}C]-glucose in tubes (6mm×30mm) placed inside stoppered scintillation vials. After incubation, 0.5ml of Hyamine was injected into the outer vessel, and islet metabolism was stopped and CO_2 liberated into the Hyamine by injection of 10μl of 0.2M-HCl into the tube containing the islets. The vessels were shaken at room temperature for a further hour to permit absorption of CO_2, and the radioactivity in Hyamine was measured by liquid-scintillation spectrometry as previously described (Ashcroft *et al.*, 1970).

Glucose utilization. Glucose utilization by islets was determined as the formation of [^{3}H]water from [5-^{3}H]glucose. The principle of this method is described below.

Incubations were carried out in 15μl of medium containing [5-^{3}H]glucose at the concentrations given and with the stated additions. The outer vials (see above) contained 0.5ml of water. After incubation, metabolism was stopped by the injection of 5μl of 0.2M-HCl into the tube containing the islets. The vessels were then incubated for a further 18–24h to allow the [^{3}H]water in the incubation tube to equilibrate with the water in the outer vial. The radioactivity in the water was then measured by liquid-scintillation spectrometry.

Control samples containing no islets were always included to allow a correction to be made for [^{3}H]water in the [5-^{3}H]glucose: in addition, samples containing known amounts of [^{3}H]water enabled estimates of the completeness of the microdiffusion of [^{3}H]water to be made. Under the conditions used approx. 75% of the [^{3}H]water was recovered.

The specific radioactivity of the medium glucose was determined by liquid-scintillation spectrometry and enzymic assay (Slein, 1963).

Insulin release. Batches of six islets were incubated in 0.6ml of medium containing albumin (2mg/ml) and other additions as stated. After incubation (2h) the medium was separated by gentle centrifugation and aspiration, diluted with phosphate–albumin buffer and stored at −20°C until assay by radioimmunoassay (Hales & Randle, 1963) with mouse insulin as standard (Coll-Garcia & Gill, 1969).

Calculation and expression of results

Insulin release rates were calculated as ng of insulin released/h per islet.

Glucose utilization rates were calculated as pmol of glucose utilized/h per islet from the formula:

$$\text{Glucose utilized (pmol)} = \frac{[^3\text{H]water formed (d.p.m.)}}{\text{sp. radioactivity of [5-}^3\text{H]glucose (d.p.m./pmol)}}$$

corrected for the recovery of [^{3}H]water.

Glucose oxidation rates were calculated as pmol of glucose oxidized/h per islet from the formula:

$$\text{Glucose oxidized (pmol)} = \frac{^{14}CO_2 \text{ formed (d.p.m.)}}{\text{sp. radioactivity of [}^{14}\text{C]glucose (d.p.m./pmol)}}$$

The specific yields of $^{14}CO_2$ from [1-^{14}C]- and [6-^{14}C]-glucose were calculated as pmol of glucose oxidized/pmol of glucose utilized and the contribution of the pentose cycle pathway to islet glucose metabolism calculated from the equation:

$$\frac{G1_{CO_2} - G6_{CO}}{1 - G6_{CO_2}} = \frac{3P}{1 + 2P}$$

where $G1_{CO_2}$ and $G6_{CO_2}$ are the specific yields of $^{14}CO_2$ from [1-^{14}C]- and [6-^{14}C]-glucose respectively

and P is the fraction of the glucose utilized that is metabolized by the pentose-cycle pathway (Katz & Wood, 1963); absolute rates of glucose metabolism via the pentose cycle were calculated as glucose utilized ×P. The pentose-cycle pathway is defined as glucose metabolized according to the overall equation:

Glucose→glucose 6-phosphate→
glyceraldehyde 3-phosphate+$3CO_2$

(Katz & Wood, 1963).

Results

Effect of glucose concentration on glucose utilization, glucose metabolism via the pentose cycle and insulin release

Rates of glucose oxidation and utilization were linear over the time-period used in these experiments. The maximum rate of glucose utilization observed was 89pmol/h per islet at 16.7mM-glucose and the half-maximum rate was achieved at approx. 6.9mM-glucose (Fig. 1). The fraction of glucose utilized that was oxidized was approximately one-third at all glucose concentrations in the range.

Insulin release was stimulated fivefold on raising the glucose concentration from 3.3 to 16.7mM in the absence of caffeine; in the presence of caffeine (5mM), a similar increase in glucose concentration elicited a 30-fold increase in the rate of insulin release but the basal insulin-secretion rate was not increased by caffeine (Table 1). Caffeine (5mM) had no effect on the rate of glucose metabolism by the pentose cycle at 3.3 or 16.7mM-glucose (Table 2). The insulin-secretory response to glucose was abolished by mannoheptulose (14.9mM) and decreased by glucosamine (20mM) (Table 1).

The contribution of the pentose cycle to glucose metabolism was small at all glucose concentrations in the range studied; at 2.8mM-glucose, 11% metabolism by the pentose cycle was observed, corresponding to 2.1pmol/h per islet; at 16.7mM-glucose the pentose cycle accounted for 1.9% of the glucose utilized, corresponding to 1.7pmol/h per islet (Fig. 1). The rate of metabolism of glucose via the pentose cycle did not change significantly over the whole range of glucose concentrations (Fig. 1).

Effects of ribose, ribitol and xylitol

In the presence of caffeine (5mM), ribose, ribitol and xylitol (20mM) had no effect on the rate of insulin release in the absence of glucose (Table 1). Insulin release at 5.6 or 20mM-glucose was not significantly affected by 20mM-ribitol or -xylitol.

There was no detectable formation of $^{14}CO_2$ from [U-^{14}C]ribose; $^{14}CO_2$ was formed from [U-^{14}C]-xylitol at a rate corresponding to 2.2pmol/h per islet (Table 3). The rate of oxidation of [^{14}C]glucose at the same concentration was 30pmol/h per islet. Ribitol and xylitol (10mM) had no significant effect on the rate of utilization of glucose (17.2mM) and glucose oxidation was unaffected by ribose (15–30mM) or xylitol (10mM) (Table 3).

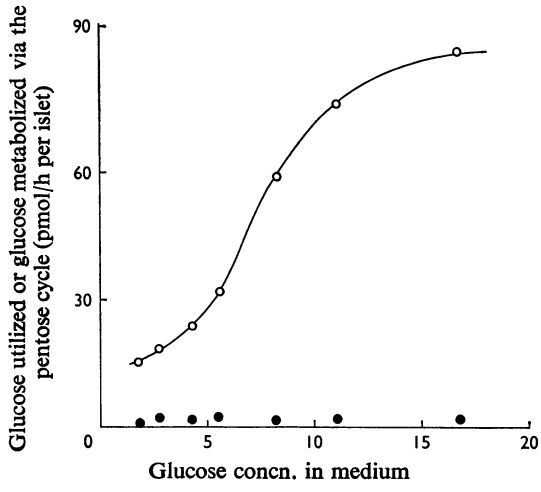

Fig. 1. *Effect of glucose concentration on glucose utilization and glucose metabolism via the pentose cycle in mouse islets*

Batches of ten islets were incubated for 2–3h at 37°C in Krebs–Henseleit bicarbonate medium containing [1-^{14}C]-, [6-^{14}C]- or [5-^{3}H]-glucose as described in the text. At each glucose concentration the rate of utilization of glucose (○) was measured, and the rate of metabolism of glucose via the pentose cycle (●) was calculated as described in the Experimental section. Each point is the mean of four or more observations.

Effects of various agents on glucose utilization and oxidation and pentose-cycle flux

The results of these experiments are given in Table 2. Glucose utilization by mouse islets was not affected by tolbutamide (0.2mg/ml), glibenclamide (1μg/ml), glucagon (5μg/ml) or cycloheximide (0.28mg/ml). Caffeine (5mM) had no effect on the rate of glucose utilization observed at an extracellular glucose concentration of 16.7mM, although at 3.3mM-glucose some inhibition of glucose utilization was observed. Mannoheptulose (14.9mM) was a potent inhibitor of glucose utilization, producing almost total suppression of glucose utilization at 8.3mM-glucose. Glucosamine (20mM) also significantly inhibited glucose utilization. Oxidation of

Table 1. *Effects of glucose, caffeine, ribose, ribitol, xylitol, mannoheptulose and glucosamine on insulin release by mouse islets*

Batches of six islets were incubated for 2h at 37°C in Krebs–Henseleit bicarbonate medium containing albumin (2mg/ml) and the additions given in the table. Insulin released into the medium was determined as described in the text. Results are given as mean ± S.E.M. with the number of batches of islets in parentheses.

Glucose concn. (mM)	Caffeine (5mM)	Other additions	Insulin release (ng/h per islet)
0	+	—	0.19 ± 0.04 (17)
0	+	Ribose (20mM)	0.21 ± 0.01 (6)
0	+	Ribitol (20mM)	0.12 ± 0.02 (6)
0	+	Xylitol (20mM)	0.16 ± 0.03 (5)
3.3	−	—	0.47 ± 0.06 (25)
3.3	+	—	0.46 ± 0.08 (27)
16.7	−	—	2.37 ± 0.20 (28)
16.7	+	—	12.67 ± 0.76 (30)
16.7	−	Mannoheptulose (14.9mM)	0.25 ± 0.05 (10)
16.7	+	Mannoheptulose (14.9mM)	0.31 ± 0.03 (12)
6.7	+	—	4.71 ± 0.85 (6)
6.7	+	Glucosamine (20mM)	1.73 ± 0.35 (6)

[1-^{14}C]- and [6-^{14}C]-glucose was inhibited by mannoheptulose, glucosamine and by iodoacetamide (0.1mM), but was not significantly affected by tolbutamide, glibenclamide, dibutyryl 3′:5′-cyclic AMP, glucagon, caffeine, theophylline (5mM) or ouabain (10μM). Phenazine methosulphate (5μM) inhibited the formation of $^{14}CO_2$ from [6-^{14}C]glucose, but not from [1-^{14}C]glucose, and increased the calculated rate of metabolism of glucose through the pentose cycle almost fourfold. Pentose-cycle flow was not affected by the other agents tested.

Phenazine methosulphate (5μM) also decreased insulin release in the presence of caffeine (5mM) and glucose (16.7mM) from 119 ± 18 to 67 ± 8pg/min per islet (mean ± S.E.M. for four observations: $P<0.05$). At higher concentrations of phenazine methosulphate, however, oxidation of both [1-^{14}C]glucose and [6-^{14}C]glucose was progressively inhibited and insulin secretion further decreased.

Discussion

Calculation of the rate of glucose metabolism via the pentose cycle

Several authors have measured yields of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose by islet tissue (Field *et al.*, 1960; Jarrett & Keen, 1966; Heinze & Steinke, 1971), and have concluded that substantial metabolism of glucose by the pentose cycle occurs. However, as emphasized by Katz & Wood (1963), estimates of pentose-cycle flux cannot be made from such data alone. Procedures have been devised for this estimation, and are discussed in detail by Katz & Wood (1963). The method used here depends on the measurement of specific $^{14}CO_2$ yields, i.e. the fraction of utilized [^{14}C]glucose oxidized to $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose. According to Katz & Wood (1963) the validity of this method depends on the following. First, the rate of metabolism of glucose by pathways that do not lead to the formation of triose phosphates should be small by comparison with its flux through the glycolytic and pentose phosphate pathways. Although this point has not been intensively investigated it seems reasonable to assume that glycogen synthesis will represent the major of such pathways and we have found that the rate of incorporation of ^{3}H from [5-^{3}H]glucose into glycogen is small (see below). Secondly, glucose 6-phosphate and fructose 6-phosphate should be in chemical and isotopic equilibrium. Although this point could not be directly ascertained it has been found that the maximum islet activity of phosphoglucoisomerase is at least 100 times greater than the maximum glycolytic flux in islets (S. J. H. Ashcroft, unpublished work) and sufficiently in excess to maintain near equilibrium of glucose 6-phosphate and fructose 6-phosphate at the concentration of glucose 6-phosphate measured in islets (Ashcroft *et al.*, 1970). Thirdly, conversion of triose phosphates into fructose 6-phosphate should not occur; this seems a reasonable assumption since the absence of fructose 1,6-diphosphatase in mouse islets has been reported (Brolin & Hellerstrom, 1967). Fourthly, an isotopic steady state should be attained; this is suggested by the linear rates of glucose oxidation and utilization that we observe. Finally, there should be no reversible exchange of ^{14}C or net synthesis of pentoses by

Table 2. *Effects of various agents on islet glucose metabolism*

Batches of ten islets were incubated in Krebs–Henseleit bicarbonate medium for 2–3 h at 37°C with radioactive glucose ([1-^{14}C]glucose, [6-^{14}C]glucose or [5-^{3}H]glucose) at the given concentrations and with additions shown. Rates of glucose utilization and oxidation were measured and rates of metabolism of glucose via the pentose cycle were calculated as described in the text. Rates of glucose utilization and oxidation are given as mean ± S.E.M. The number of observations was four except where given otherwise. * $P<0.01$.

		Islet glucose metabolism (pmol/h per islet)			
			Oxidation of		
Glucose concn. (mM)	Additions to medium	Utilization of glucose	[1-^{14}C]glucose	[6-^{14}C]glucose	Glucose metabolized via pentose cycle
3.3	—	18.9±2.8	9.0±0.6	4.6±0.1	2.4
	Tolbutamide (0.2mg/ml)	15.9±1.5	8.8±1.2	4.2±0.2	2.8
5.6	—	27.1±2.3	15.1±1.5	9.6±0.6	3.6
	Glibenclamide (1 μg/ml)	24.7±1.2	15.5±1.7	11.2±0.2	2.8
5.6	—	—	16.0±1.5	8.1±0.2	—
	Dibutyryl 3′:5′-cyclic AMP (3mM)	—	14.5±1.5	9.5±0.8	—
5.6	—	28.8±1.3	10.0±1.2	6.5±0.6	1.7
	Glucagon (5 μg/ml)	30.1±2.2	11.2±0.9	6.8±0.3	2.2
3.7	—	27.3±0.7 (5)	7.8±0.3	3.5±0.1	1.9
	Caffeine (5mM)	23.4±0.8 (5)*	7.3±0.4	3.5±0.1	1.7
16.7	—	83.5±6.1	29.6±1.1 (8)	26.9±2.5 (8)	1.3
	Caffeine (5mM)	78.7±6.3	29.2±1.5 (8)	25.9±1.9 (8)	1.6
11.7	—	—	23.3±1.9	15.7±1.4	—
	Theophylline (5mM)	—	20.2±2.7	14.7±2.0	—
10.9	—	—	21.2±1.4 (8)	14.9±1.1 (8)	—
	Ouabain (10 μM)	—	18.2±2.8 (8)	12.3±2.6 (8)	—
8.3	—	—	18.5±0.9	11.9±0.6	—
	Adrenaline (1 μM)	—	16.4±1.4	11.6±0.7	—
11.1	—	—	22.5±1.7 (5)	14.1±1.4 (5)	—
	Colchicine (1mM)	—	21.8±0.5 (5)	13.5±1.8 (5)	—
8.3	—	47.7±1.5	18.6±1.5	14.2±1.0	2.3
	Mannoheptulose (14.9mM)	5.8±4.8*	3.7±0.4*	1.6±0.1*	1.4
6.7	—	47.9±2.5	12.4±0.9 (12)	10.3±0.9 (12)	0.9
	Glucosamine (20mM)	21.4±1.5*	5.0±0.4 (12)*	2.9±0.2 (12)*	0.9
8.3	—	—	14.7±0.4*	10.7±1.0*	—
	Iodoacetamide (0.1mM)	—	8.9±1.0*	5.9±0.8*	—
11.1	—	55.8±4.5	21.7±3.1	16.6±0.9	2.7
	Phenazine methosulphate (5 μM)	42.0±2.8	22.8±3.0	5.3±0.1*	9.8
16.7	—	81.1±3.4	—	—	—
	Cycloheximide (0.28mg/ml)	82.3±5.8	—	—	—

Table 3. *Metabolism of glucose, ribose, ribitol and xylitol*

Batches of six to ten islets were incubated for 2–3 h at 37°C in Krebs–Henseleit bicarbonate medium containing the substrates given. Formation of $^{14}CO_2$ or [^{3}H]water was measured as described in the text. Results are given as mean ± S.E.M. for four observations.

Labelled substrate	Unlabelled substrate	$^{14}CO_2$ formation (pmol/h per islet)	[^{3}H]Water formation (pmol/h per islet)
[1-^{14}C]Glucose (7.2mM)	—	17.7±0.2	—
	Ribose (15mM)	16.6±1.8	—
[6-^{14}C]Glucose (7.2mM)	—	9.6±0.9	—
	Ribose (15mM)	8.8±0.9	—
[1-^{14}C]Glucose (8.3mM)	—	15.4±1.5	—
	Ribose (20mM)	13.6±1.3	—
[6-^{14}C]Glucose (8.3mM)	—	12.0±1.0	—
	Ribose (20mM)	12.8±0.6	—
[1-^{14}C]Glucose (13.9mM)	—	18.0±0.5	—
	Ribose (30mM)	18.3±0.7	—
[1-^{14}C]Glucose (10mM)	—	20.8±0.6	—
	Xylitol (10mM)	17.4±0.2	—
[6-^{14}C]Glucose (10mM)	—	16.7±0.7	—
	Xylitol (10mM)	15.6±1.3	—
[5-^{3}H]Glucose (17.2mM)	—	—	107.3± 6.6
	Ribitol (10mM)	—	98.7±10.8
	Xylitol (10mM)	—	105.2± 6.7
[U-^{14}C]Ribose (3.7mM)	—	0	—
(9.9mM)	—	0	—
(18.5mM)	—	0	—
[U-^{14}C]Xylitol (19.7mM)	—	2.2±0.1	—

transaldolase and transketolase; and either there should be complete chemical and isotopic equilibrium of triose phosphates or the rate of conversion of glucose into glycerol should be less than 20% of the rate of glucose utilization (Katz *et al.*, 1966). We have no experimental evidence bearing on these points.

The calculation of specific $^{14}CO_2$ yields requires the measurement of glucose utilization. In the present study we have used an isotopic method for determining glucose utilization. The method is based on the fact that at the triose phosphate isomerase reaction the ^{3}H of [5-^{3}H]glucose is lost to water; should this reaction not be in complete equilibrium, a further opportunity for loss of ^{3}H from this position to water occurs at the enolase reaction. Thus the rate of formation of [^{3}H]water represents the combined rates of flow through the glycolytic and pentose-cycle pathways. It does not include glucose converted into glycogen. The extent of this conversion was therefore investigated in control experiments. Islets incubated in [5-^{3}H]glucose were extracted with 30% (w/v) KOH. After the addition of carrier glycogen, the extracts were dialysed against water overnight and the radioactivity in the dialysed extracts was determined. The rate of incorporation of ^{3}H from [5-^{3}H] glucose into glycogen was found to be less than 7% of the rate of glucose utilization. We have also assessed the validity of this method for measuring glucose utilization by comparing the rate of glucose uptake determined in this way with the rate determined by direct measurement of glucose disappearance in two other tissues, namely mouse pancreas pieces and perfused rat heart: in both tissues, good agreement between the two methods was obtained.

In a previous study (Ashcroft & Randle, 1970) the total glucose-phosphorylating activity of mouse pancreatic islets was found to be 189 pmol/h per islet. Thus the maximum rate of glucose utilization measured here (89 pmol/h per islet; Fig. 1) is within the capacity of the islet glucose-phosphorylating enzymes.

Glucose utilization by mouse islets

Glucose utilization by mouse islets showed a marked dependency on extracellular glucose concentration. The rate of glucose utilization was increased sevenfold as the glucose concentration of the medium was raised from 1.8 to 16.7 mM, and there was evidence of special sensitivity to changes in glucose concentration between 5 and 11 mM (Fig. 1). The curve relating glucose-utilization rate to glucose concentration was similar in shape to those described for a number of other glucose-sensitive parameters

of islets, e.g. glucose oxidation rate and islet glucose 6-phosphate concentration (Ashcroft *et al.*, 1970), insulin release (Malaisse *et al.*, 1967), islet 6-phosphogluconate concentration (Montague & Taylor, 1970; Idahl *et al.*, 1971) and the incidence of β-cell membrane action potentials (Dean & Matthews, 1970). The only other published values for the rate of glucose utilization by mammalian islets are those of Snyder *et al.* (1970), who measured glucose uptake by isolated rat islets at 3.3 and 16.7mM extracellular glucose: the rates of 25 and 130pmol/h per islet calculable from their results are comparable with the rates of 19 and 89pmol/h per islet at these two glucose concentrations obtained in the present study (Fig. 1). In contrast with Snyder *et al.* (1970) we found no inhibition of glucose utilization by cycloheximide, an inhibitor of protein synthesis. We have previously observed that cycloheximide has no effect on glucose oxidation by mouse islets (Ashcroft *et al.*, 1970). Mannoheptulose, which inhibits glucose phosphorylation in extracts of mouse islets (Ashcroft & Randle, 1970), inhibited glucose utilization, in agreement with earlier observations on its inhibitory action on glucose oxidation (Ashcroft *et al.*, 1970). The site of action of glucosamine in inhibiting glucose utilization has not been established for this tissue; the inhibitory effect of iodoacetamide may be ascribed to inhibition of triose phosphate dehydrogenase, although additional sites of action cannot be excluded.

The pentose cycle and insulin release

When the extracellular glucose concentration was raised there was an increase in the rates of glucose utilization and insulin release, but the fraction of glucose metabolized by the pentose cycle fell, the absolute amount of pentose-cycle flux remaining constant over a wide range of glucose concentrations. Thus glucose-stimulated insulin release was not associated with any selective increase in pentose-cycle activity. This finding is in agreement with the results of Snyder *et al.* (1970). The failure of pentose-cycle flux to increase with increasing extracellular glucose concentrations may be attributable to the low K_m for glucose 6-phosphate of mouse islet glucose 6-phosphate dehydrogenase, which was considerably lower (Ashcroft & Randle, 1970) than the islet glucose 6-phosphate concentration at a low extracellular glucose concentration. The rise in islet glucose 6-phosphate concentration that occurs when the extracellular glucose concentration is raised (Ashcroft *et al.*, 1970) is thus unlikely to lead directly to an increase in the rate of metabolism of glucose 6-phosphate via glucose 6-phosphate dehydrogenase.

Montague & Taylor (1970) reported a correlation between 6-phosphogluconate concentration and insulin release that was interpreted as support for a role for the pentose cycle in insulin release from rat islets. We have observed no selective effects on pentose-cycle flux of a number of agents known to modify insulin release. Thus caffeine, which strongly potentiates glucose-stimulated insulin release from mouse islets, had no effect on the calculated pentose-cycle flux. Glucagon, tolbutamide, glibenclamide, dibutyryl 3′:5′-cyclic AMP and ouabain, which may stimulate insulin release (Coll-Garcia & Gill, 1969; Milner & Hales, 1967; Loffler *et al.*, 1969) and adrenaline or colchicine, which may inhibit insulin release (Coll-Garcia & Gill, 1968; Lacy *et al.*, 1968) were also without effect on the parameters of glucose metabolism studied here. Glucosamine inhibited glucose utilization and oxidation without affecting the rate of metabolism via the pentose cycle: mannoheptulose almost abolished glucose metabolism. Glucose-stimulated insulin release was inhibited by both these agents. Iodoacetamide inhibited the oxidation of [1-^{14}C]glucose and [6-^{14}C]glucose. These results suggest that none of these agents have any specific effect on glucose metabolism by the pentose cycle in mouse islets.

The calculated rate of flow of glucose 6-phosphate via the oxidative enzymes of the pentose cycle is some 100-fold less than the potential capacity of these enzymes as measured in extracts of islets (Ashcroft & Randle, 1970). Clearly the activity of these enzymes must be subject to control in the intact islet. One factor in this control may be the cytosol NADPH/$NADP^+$ ratio (Ashcroft & Randle, 1970). Phenazine methosulphate (5μM), which enhances the pentose pathway in some tissues by increasing the availability of $NADP^+$ (McLean, 1960), increased the calculated rate of glucose metabolism via the pentose cycle in islets by about fourfold; insulin secretion in response to 16.7mM-glucose was, however, inhibited. The effects of higher concentrations of phenazine methosulphate on the oxidation of [1-^{14}C]- and [6-^{14}C]-glucose and insulin secretion are probably attributable to actions other than on cytoplasmic NADPH, since islet ATP concentration was also progressively lowered by increasing phenazine methosulphate concentrations (L. C. C. Weerasinghe, unpublished work).

Further evidence that has been adduced in support of a role for the pentose cycle in insulin release is the stimulation of insulin secretion by various pentoses and pentose derivatives (Kuzuya *et al.*, 1966; Hirata *et al.*, 1966; Montague & Taylor, 1968). It was suggested that these compounds may be metabolized via the pentose cycle in islets, since it has been shown that the NADPH/$NADP^+$ ratio in rat islets was increased on incubation with xylitol (Montague & Taylor, 1970) and that extracts of islets catalysed the reduction of NAD^+ by ribitol or xylitol (Montague & Taylor, 1968). Other workers, however, have failed to observe stimulation of insulin secretion by pentoses or pentitols (Coore & Randle, 1964;

Lambert *et al.*, 1969; Malaisse *et al.*, 1967) and little oxidation of ribose and xylitol by rat islets was observed by Lin & Haist (1971). In the present study, there were no detectable effects on insulin release from mouse islets of ribose, ribitol or xylitol, nor did these compounds display significant metabolic activity.

We conclude from these results that in mouse islets the metabolism of glucose through the pentose cycle is unlikely to play a primary role in insulin-secretory responses. This does not rule out the possibility that changes in the concentrations of pentose cycle intermediates not immediately related to changes in the rate of flux through this pathway may be of importance; nor can we exclude a role for cofactors and intermediates produced by the pathway for the long-term functioning of the insulin synthesis and secretion processes.

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